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ANTIOXIDANT POTENTIAL OF APPLE'S PEEL EXTRACT AND ITS EFFICIENCY IN STABILIZATION OF SUNFLOWER OIL

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ABSTRACT

Three apple varieties (Chitta, Kala kolu and Golden apple) were screened for their antioxidant potential. Antioxidant activity order was established by measuring total phenolic content, reducing power and metal chelating ability. The observed order of exhibiting appreciable amount of antioxidants was Chitta apple > Kala kolu > Golden apple. Among different organic solvents, methanolic extract of all the three varieties of apple's peel produced the maximum yield. Free fatty acid (FFA), peroxide values (PV) and iodine values (IV) were used to investigate the antioxidative potential of Chitta's peel extract in stabilizing sunflower oil at two different temperatures viz. 25ºC and 55ºC during 40 days storage period. Results were statistically analysed and showed that sunflower oil containing 2400 ppm of methanolic Chitta apple's peel extract at both temperatures have maximum efficiency of stabilization and this efficiency was comparable with synthetic reference antioxidants (BHT and BHA).

Key words: antioxidant activity, apple's peel, sunflower oil, stabilization.

INTRODUCTION

Oil rancidity is a common problem which occurs due to several factors, such as temperature, formation of unsaturated fatty acids, peroxides, hydroperoxides etc. (Choe and Min, 2006). Antioxidants are the substances which have ability to control this oil rancidity by donating their hydrogens to the free radicals (Decker, 2008). In order to prevent oil rancidity, oil industries start using the synthetic antioxidants which controls this problem. However these synthetic antioxidants are carcinogenic, as well as expensive. Therefore extraction of natural antioxidants, which are present in fruits, vegetables, spices, herbs etc. is a continuous effort seen in the last few years (Sultana and Anwar, 2008; Yanishlieva and Marinova, 2001).

Apple is a fruit which is widely consumed all over the world. It is already reported in literature that peel of fruits contain more antioxidants as compared to their flesh (Escarpa and Gonzalez, 1998; Li et al., 2006). Fruit's peel is generally considered to be an agricultural waste, but in this study we utilized the peel of apples in order to stabilize commonly consumed oils during their prolonged storage. In earlier studies through accessible search we found that apple's peel containing antioxidants has already been reported, but its efficiency to stabilize sunflower oil has not been studied yet (Boyer and Liu, 2004; Sébédioo et al., 1991; van der Sluis et al., 2001; Wolfe et al., 2003).

In this present study, antioxidative potential of three varieties of apple's peel and its efficiency to stabilize the sunflower oil at 25ºC and 55ºC was investigated and the results were compared with the efficiency of common synthetic reference antioxidants i.e. Butyl-hydroxytoluene (BHT) and Butyl-hydroxyanisole (BHA).

MATERIAL AND METHODS

Three varieties of apples i.e. Chitta, Kala kolu and Golden were purchased from local

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market of Lahore, Pakistan. Refined, bleached and deodorized (RBD) sunflower oil was obtained from a Layyah, Pakistan. All the chemicals and reagents procured were of analytical grade. Synthetic reference antioxidants butyl-hydroxytoluene (BHT) and butyl-hydroxyanisole (BHA) were procured from Fluka Chemicals. All the analyses were carried out at Institute of Chemistry, University of the Punjab, Lahore, Pakistan.

Extraction in different solvent systems

All the three varieties of apples were peeled off and air dried. These dried peels were then grinded and passed through 80 mesh. 3.0 g of each variety of apple peel was taken in 150 mL of different solvent systems for extraction (80% methanol, 70% ethanol, ethylacetate and acetone). The process was repeated thrice to ensure maximum extraction. Then few drops of 0.1 M HCl were added to all residues for conversion of phenolics in free form (Singleton and Rossi, 1965; Iqbal et al., 2005). Then the combined filtrate was evaporated using rotary evaporator. Finally concentrated extracts of different solvents were obtained and their percentage yield were determined, then stored in desiccator for further analysis.

Antioxidant potential of methanolic extract of peel from three apple varieties

Total phenolic content. These contents in all apple's peel extract was determined by reported method (Singleton and Rossi, 1965). Gallic acid was used as a reference standard and results were expressed in grams of gallic acid/100 g of extract.

Chelating activity. Chelating activity of Fe^{2+} was measured by an already reported protocol (Iqbal et al., 2005). Disodium ethylene diamine tetraacetate (Na_2EDTA) was used as a reference standard and the absorbance was noted at 522 nm.

Stabilization studies of sunflower oil. For stabilization studies, methanolic Chitta apple's peel extract was taken (Iqbal et al., 2008); its different concentrations (800, 1600 and 2400 ppm) were prepared and added to preheated RBD sunflower oil (at 50ºC for 3h). Synthetic reference antioxidants (BHA and BHT) were employed at their legal limit of 200 ppm. All the treated samples (100 mL) were stored at 25ºC and 55ºC for 40 days. Similarly, control samples (without antioxidants) were also prepared and stored. All treated samples were evaluated after regular intervals of 10 days to determine peroxide, free fatty acid and iodine values (AOAC, 1990).

Statistical analysis

All the investigations were done in three replications and the resulting data were presented as mean ± standard deviation. Significant differences ($p<0.05$) were calculated by applying one way ANOVA.

RESULTS AND DISCUSSION

Effect of solvent on percentage yield (Table 1) shows the yield of apple's peel extract in different solvents. Extracts yield was ranged from 24.1% to 72.04%.

Table 1. Percentage yield of apple's peel extract

Solvent	Percentage yield (%)
Methanol	72.04 ± 1.14
Ethanol	70.33 ± 1.36
Acetone	24.10 ± 1.13
Ethylacetate	69.90 ± 1.09

The observed order of exhibiting the highest yield to extract antioxidants was methanol > ethanol > acetone > ethylacetate. These findings were also supported by previous reports (Iqbal and Bhanger, 2007; Iqbal et al., 2008). Therefore for further analysis methanolic extract was utilized.

Antioxidant potential

Total phenolic content (TPC). Literature demonstrated that most of the phenolics have antioxidative potential (Rice-Evans and Miller, 1994). TPC was determined in three varieties of apple; Kala kolu, Chitta, and Golden and results were dpicted in Table 2. TPC value showed that these different varieties of apple vary significantly ($p<0.05$). Chitta exhibited

highest (18.58 mg/g) TPC, followed by Kala kolu and Golden, respectively. Result proved that all the three varieties of apple peel exhibit antioxidant potential.

Table 2. Total phenolic content of peel extracts from different apple varieties

Varieties of apple	TPC (mg g^{-1})
Chitta	18.58 ± 1.12
Kala kolu	13.36 ± 1.36
Golden	10.84 ± 1.17

Chelating activity. Chelating activity was measured against Fe^{2+} and a large difference was observed among the varieties of apple (Table 3). The order of exhibiting the highest chelating activity was Chitta > Kala kolu > Golden. The EDTA equivalents were ranged between 431.85-519.64 mg g^{-1} of extracts for different varieties of apple. It is reported that chelation includes iron, which is an extremely reactive metal thus have ability to undergo oxidative changes in different cellular components (Ak and Gülçin, 2008; Dinis et al., 1994).

Table 3. Total chelating activity of peel extracts from different apple varieties

Varieties of apple	Chelating activity (EDTA eq.) (mg g^{-1})
Chitta	519.64 ± 13.12
Kala kolu	514.75 ± 16..36
Golden	431.85 ± 12.36

Stability of RBD sunflower oil by methanolic chitta apple's peel extract

Peroxide value (PV). Formation of peroxides and hydroperoxides due to initial oxidation occurring in fats and oils was determined by this method. Gradual increase in PVs was noticed during 40 days storage of sunflower oil at 25°C and 55°C (Table 4).

This increase was observed more effective at 55°C than at 25°C. Initially, at both temperatures the PV of sunflower oil control sample (without antioxidant) was 0.40 ± 0.14 meq kg^{-1}. After storage period of 40 days the PVs at 25°C and 55°C were 18.0 ± 1.41 meq kg^{-1} and 73.0 ± 0.41 meq kg^{-1} respectively.

Table 4. Effect of storage time on peroxide value (PV) of sunflower oil at 25ºC (A) and 55ºC (B)

Storage time (days)	SFO-Ctrl PV (meq kg^{-1})	SFO-800 PV (meq kg^{-1})	SFO-1600 PV (meq kg^{-1})	SFO-2400 PV (meq kg^{-1})	BHA-200 PV (meq kg^{-1})	BHT-200 PV (meq kg^{-1})
A. Effect of storage time on PV of sunflower oil at 25ºC						
0	0.40 ±0 .14	0.40 ±0 .14	0.40 ± 0.14	0.40 ± 0.14	0.40 ± 0.14	0.40 ± 0.14
10	3.00 ± 0.28	1.20 ± 0.28	1.00 ± 0.14	0.70 ± 0.14	0.60 ± 0.14	0.5 0± 0.14
20	8.20 ± 0.84	3.30 ± 0.28	3.10 ± 0.14	2.00 ± 0.70	1.90 ± 0.42	1.80 ± 0.42
30	14.5 ± 1.13	8.30 ± 0.14	6.60 ± 0.56	5.40 ± 0.28	5.20 ± 0.84	5.00 ± 0.84
40	18.0 ± 1.41	13.5 ± 0.28	10.0 ± 0.42	7.40 ± 0.84	8.20 ± 0.14	7.00 ± 0.14
B. Effect of storage time on PV of sunflower oil at 55ºC						
0	0.40 ± 0.14	0.40 ± 0.14	0.40 ± 0.14	0.40 ± 0.14	0.40 ± 0.14	0.40 ± 0.14
10	9.60 ± 0.56	5.60 ± 0.56	5.20 ± 0.28	4.30 ± 0.14	4.50 ± 0.28	3.90 ± 0.14
20	30.0 ± 0.56	15.0 ± 0.84	13.5 ± 0.28	7.90 ± 0.70	8.7 0± 0.84	7.80 ± 0.56
30	42.5 ± 0.70	22.7 ± 0.70	17.3 ± 0.14	10.2 ± 0.56	11.0 ± 1.13	9.00 ± 1.41
40	73.0 ± 0.41	35.3 ±0.98	20.4 ± 1.41	12.3 ± 0.14	14.0 ± 1.41	11.5 ± 0.56

Same gradual increase in PVs of apple's peel extract and synthetic reference antioxidants i.e. BHA and BHT was observed. But, as compared to the control sample, the PV was reduced at both temperatures during storage. Methanolic chitta apple's peel extract at 2400 ppm showed no distinct difference with synthetic reference antioxidants.

The observed order at both temperatures was control > SFO-800 > SFO-1600 > SFO-BHA > SFO-2400 > SFO-BHT. This result is

similar with the findings of (Kiyomi and Yasuko, 1995; Noor and Augustin, 1984).

Free fatty acid value (FFA). Deterioration of fats and oils due to oxidative process across double bond in triglyceride molecule was already reported in literature (Frega et al., 1999). This deterioration can be determined in fats and oils by measuring free fatty acid value. Table 5 show the gradual increase in free fatty acid value at 25°C and 55°C, which confirms the formation of fatty acids that ultimately undergo into deterioration. The increase was observed more pronounced at 55°C relative to 25°C. Initially, the FFA value of sunflower oil control sample (without antioxidant) was 0.103 ± 0.02 %. After 40 days storage, the FFA value at 25°C and 55°C were found to be 0.520 ± 0.07 % and 0.962 ± 0.06 % respectively. It is obvious from the results that by adding chitta apple's peel extract and synthetic reference antioxidants, the development of rancidity in sunflower oil get retarded during storage.

At both temperatures, 2400 ppm of methanolic chitta apple's peel extract showed best efficiency to prevent the oil from becoming rancid. This concentration of apple's peel extract was comparable with synthetic reference antioxidants (BHA and BHT).

The observed order at both temperatures was control > SFO-800 > SFO-1600 > SFO-BHA > SFO-2400 > SFO-BHT. Statistical analysis proved that FFA value of sunflower oil varied significantly ($p<0.05$) by adding apple's peel extract and synthetic reference antioxidants.

Table 5. Effect of storage time on free fatty acid value (FFA) of sunflower oil at 25ºC (A) and at 55ºC

Storage time (days)	SFO-Ctrl FFA (% oleic acid)	SFO-800 FFA (% oleic acid)	SFO-1600 FFA (% oleic acid)	SFO-2400 FFA (% oleic acid)	BHA-200 FFA (% oleic acid)	BHT-200 FFA (% oleic acid)
A. Effect of storage time on FFA of sunflower oil at 25ºC						
0	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02
10	0.209 ± 0.01	0.176 ± 0.03	0.153 ± 0.04	0.128 ± 0.04	0.133 ± 0.03	0.119 ± 0.06
20	0.323 ± 0.07	0.256 ± 0.06	0.186 ± 0.04	0.138 ± 0.06	0.146 ± 0.06	0.132 ± 0.07
30	0.398 ± 0.06	0.332 ± 0.07	0.267 ± 0.01	0.176 ± 0.01	0.186 ± 0.05	0.163 ± 0.06
40	0.520 ± 0.07	0.402 ± 0.04	0.362 ± 0.03	0.225 ± 0.02	0.233 ± 0.07	0.202 ± 0.05
B. Effect of storage time on FFA of sunflower oil at 55ºC						
0	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02	0.103 ± 0.02
10	0.256 ± 0.03	0.203 ± 0.03	0.188 ± 0.04	0.133 ± 0.03	0.132 ± 0.01	0.123 ± 0.03
20	0.438 ± 0.06	0.269 ± 0.06	0.222 ± 0.07	0.163 ± 0.07	0.186 ± 0.03	0.158 ± 0.06
30	0.693 ± 0.07	0.452 ± 0.07	0.309 ± 0.01	0.202 ± 0.03	0.236 ± 0.05	0.192 ± 0.07
40	0.962 ± 0.07	0.639 ± 0.04	0.402 ± 0.02	0.309 ± 0.02	0.333 ± 0.02	0.293 ± 0.04

Iodine value (IV). Iodine value determines the degree of unsaturation in the oil. The increase in iodine value shows the increase in degree of unsaturation in the oil (Kerrihard et al., 2015). Table 6 show the iodine value (IV) of refined sunflower oil at 25ºC and 55ºC during storage. A gradual decrease was observed in iodine value at 25°C and 55°C during 40 days storage. This decrease was more prominent at 55°C compared to 25°C. Firstly, the iodine value (IV) of all the samples at both temperatures remained the same, but with time a decrease was observed which indicates the degree of unsaturation. Treated sunflower oil samples at both temperatures showed less decrease in IV as compared to the control (without antioxidant) samples. It is evident from the results that the iodine value of treated sunflower oil samples with methanolic Chitta apple's peel extract (2400 ppm) and synthetic reference antioxidant (BHA) were comparable, but slightly lower than BHT.

Table 6. Effect of storage time on iodine value (IV) of sunflower oil at 25ºC and at 55ºC

Storage time (days)	SFO-Ctrl (IV)	SFO-800 (IV)	SFO-1600 (IV)	SFO-2400 (IV)	BHA-200 (IV)	BHT-200 (IV)
A. Effect of storage time on IV of sunflower oil at 25ºC						
0	298 ± 1.18	298 ± 1.18	298 ± 1.18	298 ± 1.18	298 ± 1.18	298 ± 1.18
10	220 ± 1.20	269 ± 1.35	271 ± 1.20	278 ± 1.05	280 ± 1.14	282 ± 1.13
20	171 ± 1.35	210 ± 1.16	217 ± 1.29	250 ± 1.23	254 ± 1.16	257 ± 1.10
30	143 ± 1.56	178 ± 1.17	190 ± 1.22	104 ± 1.21	206 ± 1.39	208 ± 1.35
40	114 ± 1.20	152 ± 1.13	163 ± 1.05	178 ± 1.12	180 ± 1.17	183 ± 1.14
B Effect of storage time on IV of sunflower oil at 55ºC						
0	298 ± 1.18	298 ± 1.18	298 ± 1.18	298 ± 1.18	298 ± 1.18	298 ± 1.18
10	240 ± 1.11	262 ± 1.20	268 ± 1.10	271 ± 1.02	273 ± 1.17	275 ± 1.26
20	180 ± 1.29	208 ± 1.16	215 ± 1.17	241 ± 1.27	245 ± 1.21	250 ± 1.19
30	133 ± 1.31	169 ± 1.22	177 ± 1.25	191 ± 1.13	193 ± 1.20	197 ± 1.20
40	108 ± 1.17	148 ± 1.13	153 ± 1.20	164 ± 1.16	166 ± 1.17	178 ± 1.16

CONCLUSIONS

It is concluded that antioxidative potential of Chitta apple's peel extract made it to be a better natural antioxidant as compared to synthetic reference antioxidants. Peroxide, free fatty acid and iodine values proved that 2400 ppm of Chitta apple's peel extract had better efficiency to stop deterioration in oils as compared to BHA, but slightly lower than BHT. Therefore, it is suggested to use apple's peel extract as a good effective antioxidant in various additives and also in oils during their prolonged storage.

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